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Astrocytes in neuroendocrine systems: An overview

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Abstract

A class of glial cell, astrocytes are highly abundant in the CNS. In addition to maintaining tissue homeostasis, astrocytes regulate neuronal communication and synaptic plasticity. There is an ever-increasing appreciation that astrocytes are involved in the regulation of physiology and behaviour in normal and pathological states, including within neuroendocrine systems. Indeed, astrocytes are direct targets of hormone action in the CNS, via receptors expressed on their surface, and are also a source of regulatory neuropeptides, neurotransmitters, and gliotransmitters. Furthermore, as part of the

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neurovascular unit, astrocytes can regulate hormone entry into the CNS. This review is intended to provide an overview of how astrocytes are impacted by and contribute to the regulation of a diverse range of neuroendocrine systems: energy homeostasis and metabolism, reproduction, fluid homeostasis, the stress response, and circadian rhythms.

Astrocytes are a type of glial cell abundant in the central nervous system (CNS). Although historically known for their role in maintaining tissue homeostasis, there is a growing recognition that astrocytes actively contribute to the regulation of physiology and behaviour via modulation of neuronal circuits. The goal of this review is to provide an overview of the emerging contribution of astrocytes in neuroendocrine function, highlighting other recent in-depth reviews and key studies for those wishing to explore specific areas further. Related to astrocytes, tanycytes are a specialised glial-cell type lining the base of the third ventricle in the hypothalamus. While our understanding of the importance of tanycytes in neuroendocrine function is evolving, in the interest of focus they will not feature in this article and we refer the readers to other recent reviews in this area (1, 2). Although the pituitary gland contains glial cells with morphological similarities to astrocytes, namely pituicytes (3), these will not feature in this review.

An overview of astrocyte function

Astrocytes are found throughout the CNS, but the ratio of astrocytes to neurons varies depending on the brain region. For a comprehensive overview of the cellular physiology of astrocytes we refer the readers to the recent review by Verkhratsky and Nedergaard (4). Astrocytes utilise variations in intracellular Ca^{2+} as their main ionic signalling modality and, in contrast to neurons, are not directly electrically excitable.

Astrocytes regulate tissue homeostasis within the brain including supplying energy substrates to neurons, maintaining pH and ionic balance. Importantly, they also functionally modulate neuronal activity in several ways (4): 1) regulation of ion and neurotransmitter availability (via modulating synthesis, uptake, and recycling); 2) releasing gliotransmitters such as ATP/adenosine, lactate and D-serine; 3) modulating blood brain barrier (BBB)

permeability and thus controlling entrance and efflux of substances to/from the brain; 4) providing a plastic physical barrier to extra-synaptic neuronal communication through ensheathment of synapses, and 5) regulating synaptic stability. Additionally, astrocytes can regulate neuronal growth (5) and the formation and elimination of synapses during brain development (6-9).

Astrocytes are heterogeneous

Astrocytes are heterogeneous in both their morphology and function (10, 11). These differences can be seen both between and within different regions of the brain. Astrocyte heterogeneity arises partly during development (12); however, plasticity allows astrocytes to change in both form and function in response to physiological and pathological changes within the brain, including in response to aging (13). For example, during brain damage and neurological disease, astrocytes undergo morphological and functional changes termed reactive astrogliosis (14). This process has many roles in neuropathology: forming “glial-scars” which separate healthy and diseased tissue (15), enabling phagocytic activity in astrocytes (16), and stimulating astrocytes to become mediators which regulate immune cell recruitment in the CNS (15).

Transcriptional analyses indicate that neurodegenerative diseases and aging are characterised by the formation of “A1-like” reactive neuroinflammatory astrocytes, which have lost neurotrophic capacity and instead contribute to death of neurons and oligodendrocytes via release of toxic factors (17, 18). In contrast, ischemic injury is characterised by a predominance of an “A2-like” phenotype amongst the reactive astrocytes, in which astrocyte expression of genes which promote neuronal growth, survival, and synapse repair are increased (19). Understanding astrocyte heterogeneity and the creation of functional classifications is an evolving area and, as is the case with peripheral immune cells (20), phenotypes are likely to be on a plastic continuum depending on variations in microenvironmental conditions within the CNS.

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Glial-fibrillary acidic protein (GFAP) is an intermediate filament protein and commonly used cellular marker astrocytes throughout the brain, although it is also expressed in tanycytes and ependymal cells (21). The GFAP is promotor used experimentally to drive targeted genetic-modification of astrocytes *in vitro* and *in vivo*. While GFAP levels are increased in reactive astrocytes and it is used histologically to visualise morphological changes in astrogliosis, it is important to note not all astrocytes, including those in the hypothalamus, express detectable levels of GFAP. Indeed, when GFAP-immunoreactivity is used to quantify changes astrocyte number in response to different experimental or physiological manipulations, it is not necessarily clear whether additional numbers of GFAP-immunoreactive cells reflect astrocyte proliferation or the rise of detectable levels of GFAP pre-existing astrocytes. Other frequently used markers of astrocytes include GLAST, GLT-1 and S100B, although the relative specificity of these is age and brain region specific. Aldehyde dehydrogenase 1 family member L1 (Aldh1L1) is emerging as a specific pan-astrocyte marker (22) and the Aldh1L1 promotor is increasingly being used to facilitate genetic modification of astrocytes. Moving forward, use of both GFAP and Aldh1L1 expression (gene and protein) will give a more holistic picture of astrocyte form and function than assessment of GFAP levels alone.

The remainder of this review will be focused on evidence for astrocytic involvement in neuroendocrine regulation, largely focusing on hypothalamic astrocytes, but touching on astrocytes in other brain regions where pertinent information is available.

Astrocytes are key players in neuroendocrine regulation

Astrocytes are direct targets of hormone action via receptors expressed on their surface. As such, hormones can impact astrocyte morphology and function including regulating neurotransmitter availability, and release of gliotransmitters and active metabolic intermediates, which can in turn influence neuronal communication. Astrocytes are also a source of bioactive signalling molecules in the brain: neuropeptides, neurosteroids, and hormones (via direct synthesis or processing of precursors, by enzymes such as deiodinase 2, following their uptake into astrocytes by specific transporters). As part of the

neurovascular unit, astrocytes can also directly influence BBB permeability and thereby may regulate entrance and efflux of hormones, and other key neuroendocrine modulators such as nutrients and cytokines, from the brain (23). A summary of the proposed mechanisms by which astrocytes regulate the activity of neuroendocrine systems is illustrated in **Figure 1**.

Astrocytes impact the activity of a variety of neuroendocrine systems, introduced individually below.

Astrocytes and the neuroendocrine regulation of energy homeostasis and metabolism

Food intake and body weight

The study of astrocytes in the central control of energy homeostasis has been a growing field in recent years. The discovery that consumption of a high-fat diet increases mRNA for known inflammatory molecules in the rat hypothalamus drew attention to hypothalamic inflammation as an area of study (24). Within the hypothalamus nuclear factor kappa B (NF- κ B) is activated in diet-induced obesity (DIO) and virally-mediated genetic activation of IKK β , a kinase known to activate NF- κ B, in the mouse hypothalamus is sufficient to induce insulin and leptin resistance (25). Moreover, DIO increases glial-fibrillary acidic protein (GFAP; a commonly used marker of astrocytes) gene expression and astrocytic ensheathment of proopiomelanocortin neurons (POMC) in the arcuate nucleus (ARC), consequently decreasing the number of synaptic inputs to POMC neurons; thus, reducing neural activity critical for the inhibition of food intake (26). Following this seminal study, other groups have demonstrated that DIO in rodents is accompanied by an increase in GFAP expression (both at the gene and protein level) and altered morphology of astrocytes in the ARC and other hypothalamic nuclei (27, 28). This astrogliosis is also seen in melanocortin-4 receptor deficient mice, which become obese on standard chow diet, due to excess intake (27), suggesting that the gliosis can occur because of obesity in the absence of high-fat diet consumption. Returning DIO mice to a standard chow diet results in weight loss and reversal of the astrogliosis (29), highlighting the plastic nature of this response. The physiological significance of these changes is reinforced by studies indicating that inducing inflammatory signalling in astrocytes results in obesity, while preventing inflammation preserves astrocytic plasticity protecting against DIO (30). Supporting this supposition, brain-wide

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deletion of astrocyte IKK β during the induction of DIO is protective against hypothalamic inflammation and prevents subsequent weight gain (31). While supportive evidence for obesity-associated glial changes from human studies is limited, there is a positive correlation between body mass index and signatures of hypothalamic gliosis, measured by hyper-intensity in magnetic resonance imaging scans (28). Together, these studies implicate astrocyte inflammatory processes, plausibly initiated by IKK β , in the development and maintenance of DIO possibly via a loss of astrocyte structural plasticity.

The response of hypothalamic astrocytes in rodents to a high-fat diet appears to be biphasic: there is an initial increase in expression GFAP and pro-inflammatory markers during the first 24-72h on a high-fat diet, which then transiently subsides before returning after 14 days on the diet (28, 32, 33). The first phase of astrocyte activation may occur independently of leptin resistance (32). Inhibition of astrocyte NF- κ B mediated inflammatory signalling during the initial phase of high-fat diet-induced astrogliosis abolishes changes in GFAP expression and augments high-fat diet-induced binge eating in mice (33). This has led to the suggestion that the initial phase of astrogliosis is partly a homeostatic or allostatic physiological response to maintain energy balance while the second inflammatory phase is a pathophysiological manifestation of prolonged deviation from energy homeostasis. Further evidence for a role of astrocytes in the physiological response to energy imbalance comes from experiments where mice are pushed out of homeostasis into negative energy balance by fasting. In common with acute energy excess associated with feeding a high-fat diet, fasting impacts hypothalamic astrocyte signalling (30, 34, 35). Together these studies indicate that hypothalamic astrocytes are sensitive to acute deviations in energy homeostasis.

Astrocytes can directly sense and respond to appetite-modulating hormones via receptors expressed on their surface. Astrocytes neighbouring the third ventricle (3V) that are sensitive to peripheral cues express leptin receptors (36). Diet-induced and genetic obesity increases the number of leptin receptor-expressing astrocytes in the ARC and dorsomedial nucleus (DMN) of the hypothalamus (37, 38). Functional studies indicate that chronic (14

day) intracerebroventricular (ICV) delivery of exogenous leptin increases hypothalamic expression of GFAP and vimentin and increases astrocyte process length. The same treatment reduces expression of synaptic proteins, suggesting increased glial ensheathment of neurons obstructing synapse formation, which may impair integration of feeding cues by these neurons (39). ICV delivery of leptin for 14 days also decreases the expression of astrocyte glucose and glutamate transporters in the hypothalamus, possibly leading to elevated synaptic glutamate (35). A study utilising a mouse model where leptin receptors were selectively deleted from astrocytes in adult mice revealed reduced ensheathment of ARC neurons by astrocytes lacking the leptin receptor (40). In accordance with this, these mice showed reduced sensitivity to leptin and increased sensitivity to ghrelin. These results suggest leptin receptors on astrocytes are crucial for anatomical wiring of the ARC melanocortin circuits critical for the regulation of energy homeostasis and that this wiring ensures appropriate responses to endocrine signals that modulate food intake. A second mouse model with developmental astrocyte-specific deletion of leptin receptors showed an increased body-weight and fat-mass gain on a high fat diet, accompanied by greater hypothalamic leptin resistance and increased gliosis compared with wild-type controls (41). Astrocyte sensing of leptin also appears to be critical during hypothalamic development (42).

Like leptin, ICV treatment with the orexigenic hormone ghrelin regulates the expression of a number of proteins expressed by hypothalamic astrocytes including structural proteins (GFAP and vimentin) and transporters (glucose and glutamate transporters) (43). *In vitro* primary mouse astrocytes, primary rat astrocytes and the C6 astrocytoma cell line are responsive to ghrelin, while glia cultured from mice lacking the growth-hormone secretagogue receptor 1A (GHSR-1A) do not, suggesting some of the effects of ghrelin may be due to direct activation of astrocyte GHSR-1A (43-45). This is supported by immunohistochemistry which has identified GHSR-1A co-localised with GFAP in the rat hypothalamus (43). Together these studies provide evidence that astrocytes are direct sensors of endocrine signals regulating energy balance, and likely mediate, at least in part, some of the physiological effects of these hormones in the CNS.

The application of opto- and chemo-genetic technologies for manipulation of ARC astrocyte signalling has provided conflicting results with respect to astrocytic regulation of feeding behaviour. In 2015, Yang and colleagues reported that chemogenetic activation of Ca^{2+} signalling in ARC astrocytes caused a decrease in both nocturnal feeding and ghrelin-induced food intake while enhancing the satiating effect of leptin. Their work suggests this was mediated by astrocyte-derived adenosine inhibiting agouti-related peptide (AgRP) neurons via activation of pre-synaptic adenosine A1 receptors (46). This was supported by follow-up study from the same group, which showed that optogenetic-mediated activation ARC astrocyte signalling reduced food intake (47). However, in contrast a second independent research group, employing a similar chemogenetic methodology, found that activating Ca^{2+} signalling hypothalamic astrocytes increased food intake during the dark phase by excitation of AgRP neurons (34). The discrepancies in these results may be due to technical differences (e.g. injection of virus, dose of chemogenetic ligand) or may represent a state-dependent effect of astrocyte activation on feeding behaviour. As such, much remains to be learnt about the contribution of astrocytes to hypothalamic control of feeding.

Although not as widely studied in this context, astrocytes outside the hypothalamus may also play a role in regulating food intake given that they can control some consummatory behaviours. For example, activation of nucleus accumbens (NAc) core astrocytes reduces the self-administration of cocaine after reinstatement via an increase in synaptic glutamate (48). The process of cocaine self-administration and extinction appears to render NAc core astrocytes hypoactive, suggesting they play a role in addiction (49). Given that NAc neurons are thought to be involved in driving the motivation to feed (50), astrocyte regulation of transmission in this circuit may alter feeding, although this remains to be investigated. A second population of extra-hypothalamic astrocytes shown to influence food intake are those in the nucleus of the solitary tract (NTS). NTS astrocytes appear to play a role in glucagon-like peptide (GLP-1) mediated-satiety since these cells show increases in intracellular Ca^{2+} in response to the GLP-1 receptor agonist exendin-4 in an *ex vivo* rat brain slice. In rats, delivery of exendin-4 to the NTS reduces food intake, an effect that is abolished by pre-treatment with the astrocyte metabolic inhibitor fluorocitrate (51). The exact

mechanism for this effect remains unclear as GLP-1 receptors were not observed on astrocytes in mice expressing green fluorescent protein in GLP-1 receptor containing cells (52).

To conclude, astrocytes in the hypothalamus are responsive to short-term and prolonged deviations in energy homeostasis. These cells can directly sense appetite-regulating hormones and adjust morphology, expression of transporters, and potentially release of gliotransmitters accordingly, to alter the firing of neurons that control food intake. Extra-hypothalamic astrocytes may play a role in food intake which will be an emerging area for future research. A schematic summary of the current understanding of how astrocytes may impact feeding behaviour can be found in **Figure 2**.

Glucose homeostasis

Glycaemia regulation is largely controlled by the pancreas, principally by glucose-dependent insulin secretion from pancreatic β -cells through Ca^{2+} dependent exocytosis¹. In health, insulin secretion is largely sufficient to control blood glucose within the healthy range (4-7mM glucose). When β -cells are compromised, such as in type 1 diabetes, or when insulin action is insufficient to adequately control glucose levels, such as during early type 2 diabetes, other tissues, such as the brain, play a more prominent role.

In the CNS, blood glucose is mainly regulated by the hypothalamus, with the hindbrain also playing an important role. POMC, AgRP, and neuropeptide Y (NPY) neurons of the ARC and steroidogenic factor-1 (SF-1) neurons of the ventromedial nucleus of the hypothalamus (VMN) all modulate glucose homeostasis through regulation of food intake and pancreatic hormone release (53-55).

Given that astrocytes are near the cerebral blood supply and directly interact with pericytes which regulate vascular tone, it is likely that astrocytes detect changes in glucose levels before neurons. Over the past decade several studies have demonstrated that astrocytes detect changes in glucose availability, and direct manipulation of astrocyte function alters glycaemia and/or glucose tolerance. For example, glucose insufficiency induced by 2-deoxyglucose (2-DG) increases intracellular Ca^{2+} in hindbrain astrocytes (56). This astrocyte activation is required for the stimulation of adjacent catecholamine neurons, indicating that astrocytes couple glucose availability to neuronal activation (56). Catecholamine neurons stimulate the release of adrenaline, contributing to the counterregulatory response (CRR) to hypoglycaemia, suggesting astrocytes play a role in the CRR. Rats exposed to three days of recurrent insulin-induced hypoglycaemia have reduced hypothalamic expression of the main astrocytic glutamate transporter GLT-1. This correlates with reduced glutamate uptake in primary cultured rat astrocytes, and to glutamatergic neurotransmission failure *in vivo*, attenuating activation of the CRR (57). Further supporting an astrocytic role in full activation of the CRR to hypoglycaemia, loss of the brain glucose transporter GLUT2 causes loss of the glucagon response to hypoglycaemia in mice. Rescue of GLUT2 levels specifically in GFAP-expressing astrocytes is sufficient to restore the glucagon response to hypoglycaemia, suggesting that astrocytic glucose sensing is essential to maintaining glucose homeostasis (58). In addition, stimulation of the CRR following fourth ventricular (4V) administration of 2-DG is ablated following pre-treatment with glial metabolic inhibitor fluorocitrate, indicating that astrocytes neighbouring the 4V are also required for activation of the CRR. These data suggest changes in hypothalamic and hindbrain astrocyte function are required for full activation of the CRR to hypoglycaemia.

In addition to astrocytic GLUT2 restoring the CRR as discussed above, there is further evidence that astrocyte function is directly regulated by glucose availability. Insulin receptor deletion from hypothalamic astrocytes in mice reduces astrocytic intracellular glucose levels and astrocytic ramification (59), reducing the physical contact between neurons and astrocytes. Sensitivity to changes in glucose availability and subsequent communication of POMC neurons is also reduced. This induces glucose intolerance, likely mediated by reduced brain glucose transporter GLUT1 expression (59). Interestingly, in a separate study,

overexpression of GLUT1 in GFAP-containing cells was sufficient to rescue diabetic hyperglycaemia induced by STZ (streptozotocin) administration (60). Taken together, these data suggest that increased or decreased glucose delivery into hypothalamic astrocytes (via GLUT1) is key in the central sensing of glucose and subsequent regulation of peripheral glucose levels.

In the CNS glycogen is stored predominantly in astrocytes (61). In astrocytes, as in the periphery, glycogen synthesis is dependent upon insulin receptor activation through the PI3K/Akt pathway to increase intracellular glycogen storage during glucose sufficiency. Although the glycogen content of astrocytes is low compared to liver and skeletal muscle, it is biologically meaningful. Glycogen breakdown by glycogenolysis is required to sustain neural activity during glucose deprivation (62). Moreover, astrocytic glycogen supercompensation is postulated to contribute to CRR failure following recurrent hypoglycaemia. For example, in mice hypothalamic glycogen content doubles in response to repeated once daily brain injection of 2-DG to simulate recurrent hypoglycaemia (63). On recovery from insulin-induced hypoglycaemia in mice, brain glycogen content has been reported to increase by 25% over basal levels, suggesting short term glycogen supercompensation. This may be a species-specific phenomenon as little or no glycogen supercompensation has been described in rat hypothalamus after hypoglycaemia (64). However, some glycogen supercompensation has been observed in cortex, striatum and hippocampus following acute hypoglycaemia in rats (65). Whether glycogen supercompensation following acute/recurrent hypoglycaemia occurs in humans with diabetes remains uncertain (66).

In both STZ-induced diabetes and the non-obese diabetic (NOD) mouse models increased hippocampal astrogliosis has been observed reflected in both increased number of GFAP-immunoreactive cells and increased cellular area (67). Conversely, in rats with poorly-controlled diabetes (STZ-induced) the number of hypothalamic GFAP-immunoreactive cells decreased within 6 weeks of poor glucose control, likely mediated by increased astrocytic cell death (68, 69). As discussed above, in humans and in rodent models, obesity which is often concurrent with elevated glucose levels, also causes hypothalamic gliosis and inflammation (28).

Glucose intolerance driven by high-fat diet challenge also has an astrocytic component. For example, astrocyte-specific inducible knockout of the NF- κ B activating molecule IKK β improves glucose intolerance caused by DIO, restoring glucose handling and insulin sensitivity in addition to reducing astrogliosis in the medial basal hypothalamus/ARC (30). Additionally, astrocyte-specific constitutive activation of IKK β causes glucose intolerance in chow fed mice through a mechanism involving increased extracellular GABA preventing the release of the growth hormone brain derived neurotrophic factor (BDNF) (30). Together these data suggest that inflammatory astrogliosis mediated by the NF- κ B transcription factor dysregulates glucose homeostasis through increased GABA and reduced BDNF release.

In summary, astrocytes play a crucial role in maintaining glucose homeostasis by regulating the hypoglycaemic CRR through direct glucose sensing and conversely synthesising and storing glycogen when sufficient glucose is available. Beyond this, astrocytes activate neuronal networks implicated in endocrine glucose regulation through NF- κ B activation, and glutamate and GABA signalling in direct response to availability of glucose, insulin and glucagon. A schematic of the current understanding of how astrocytes may impact systemic glucose homeostasis can be found in **Figure 3**.

Thyroid hormone axis

The hypothalamic-pituitary-thyroid (HPT) axis is a key neuroendocrine feedback system regulating growth, development, and metabolism, which when impaired also impacts reproductive capacity and cognition. Thyrotropin releasing hormone (TRH) neurons in the hypothalamus project to the anterior pituitary thyrotropes and stimulate the release of thyrotropin/thyroid stimulating hormone (TSH) which in turn stimulates thyroxine (T4), and to a lesser degree 3,3',5-triiodothyronine (T3), from the thyroid gland. Plasma T4 and T3, and T3 locally produced in the brain, provide negative feedback regulating the production of TRH and TSH. Limited evidence, largely from *in vitro* studies, suggests that astrocytes may be direct targets of action of TRH via receptors expressed on their surface (70-72) but the physiological relevance of this remains unclear.

Astrocytes express a variety of thyroid hormone transporters (73). Whether these cells actively participate in transport of thyroid hormones into the brain across the BBB is uncertain (74), but once in the CNS, thyroid hormones are taken up into astrocytes and tanycytes which mediate local conversion of T4 to T3 by type-2 deiodinase 2 (D2) (75, 76). The half-life of D2 is regulated by the presence of T4: when T4 levels are low the half-life of D2 is increased and *vice versa* (77). However, within the hypothalamus T4-induced D2 regulation is minimal and D2 activity is preserved even when T4 levels are high, enabling the hypothalamus to maintain higher sensitivity to T4 (78). Within the hypothalamus, endocrine action of plasma T3 and paracrine action of locally produced T3 on TRH neurons are part of the negative feedback loop which inhibits HPT axis activity. In mice, genetically mediated germ-line deletion of the deiodinase 2 gene (*DIO2*) specifically from GFAP-expressing cells (encompassing a population of astrocytes) reduces D2 activity in the cortex, hippocampus and cerebellum, but not the hypothalamus indicating that tanycytic D2 and not astrocytic D2 is likely responsible for mediating T4 to T3 conversion within this brain region (79). This was further supported by the fact that loss of D2 in GFAP-expressing cells did not result in HPT-axis dysfunction or alterations in the morphology of the thyroid. However, despite no overt changes in growth curves, body composition or feeding behaviour, these animals show subtle changes in whole body metabolism consistent with changes in substrate utilization suggesting a contribution of D2-generated T3 in the brain, mostly likely the hypothalamus and/or brainstem, in influencing systemic energy homeostasis (80).

Astrocyte function is directly modulated by thyroid hormones, which in turn can modulate neuronal activity. Exposure of primary human astrocytes in culture to T3 stimulates fatty-acid oxidation and ATP production (81). Neurotrophin-3 and basic fibroblast growth factor (FGF-2) have been implicated as factors released from astrocytes in response to T3 which impact neuronal activity (82). In addition to modulating gliotransmitter release, *in vitro* studies demonstrate that T3 enhances glutamate uptake into astrocytes by increasing the activity of astrocyte glutamate transporters GLT-1 and GLAST (83), which could in turn impact communication between neurons by altering the glutamate availability in the synaptic cleft. Together these data suggest that in addition to contributing to controlling thyroid hormone signalling in the brain by regulating local T3 levels, autocrine or paracrine activity of T3 on astrocytes in the CNS may modulate neuronal communication.

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It remains to be determined whether astrocytes form part of the negative feedback loop that regulates HPT-axis function by modulating activity of TRH neurons. Furthermore, it is unclear how the effects of T3 on astrocyte activity are mediated, as the presence of thyroid hormone receptors in astrocytes is controversial (84) so more work is needed in this area.

Astrocytes and the neuroendocrine regulation of reproductive function

Hypothalamic-pituitary-gonadal (HPG) axis

Sitting at the top of the hypothalamic-pituitary-gonadal (HPG) axis, gonadotropin releasing hormone (GnRH; also known as luteinising hormone releasing hormone [LHRH]) neurons are master regulators of the neuroendocrine control of reproduction. GnRH-secreting neurons originate in the nasal placode. During prenatal development their cell bodies migrate to the hypothalamic preoptic area (POA) and organum vasculosum laminae terminalis (OVLT). GnRH neurons project to the median eminence where they secrete GnRH into the hypophyseal portal circulation. GnRH acts at receptors on gonadotroph cells in the pituitary which secrete luteinising hormone (LH) or follicle stimulating hormone (FSH) depending on the pulsatile pattern of GnRH release. Appropriate pulsatile release of these hormones is essential for the initiation and maintenance of reproductive function. These hormones act on the gonads which in turn release their own hormones which provide negative feedback to the hypothalamus, completing the axis.

Synchronised GnRH neuronal firing has been seen *in vitro* and is proposed to be essential for the pulsatile GnRH secretion required for LH release (85). *In vivo*, GnRH neurons are intermingled with other hypothalamic cells and therefore direct gap junctional connection between neighbouring GnRH neurons is unlikely. This raises the possibility that perhaps synchrony arises from gap junctions between GnRH neurons and astrocytes, which have been reported to be formed of connexin (Cx) 43, 26 and 32 proteins (86). Indeed, oestrogen and/or progesterone administration increases the amount of Cx43 in the POA of female but not male rats suggesting sexually dimorphic function of astrocyte gap junctions (87). The premise that astrocytes may be key players in the coordination of GnRH neuron synchrony is

supported by recent data indicating that GFAP, Cx43 and GnRH closely associate in the mouse median eminence. In addition, in cultured embryonic mouse nasal placode explants, pharmacological Cx43 gap junction blockade reduces both GnRH neuron synchronicity and GnRH secretion (88). Furthermore, female Cx43^{+/-} mice show altered reproductive behaviour and disrupted oestrus cycles, providing indirect *in vivo* evidence for a potential role of astrocyte-neuronal communication in the regulation of reproductive function (87).

In addition to connexins, other mediators of neural cell interaction have been implicated in regulating activity of GnRH neurons. Polysialylated neural cell adhesion molecule (PSA-NCAM) promotes cell movement by hindering cell adhesion and has been investigated in relation to the structural plasticity of GnRH neurons. Removing the PSA from NCAM in the median eminence of cycling female rats appears to alter the structural interaction between astrocytes (GFAP-immunoreactivity) and GnRH neurons (GnRH-immunoreactivity) (89); however, this study did not report any disruption of the oestrus cycle in these mice, so the physiological impact remains unclear. Another adhesion-related protein expressed by hypothalamic astrocytes is receptor-like protein tyrosine phosphatase β (RPTP- β), a receptor which adheres to the cell adhesion molecule contactin (90). RPTP- β expression increases in the hypothalamus of female mice immediately prior to puberty suggesting a role for contactin (GnRH neurons) to RPTP- β (astrocytes) adhesion in structural plasticity (90). A further adhesion molecule, namely synaptic cell adhesion molecule 1 (SynCAM1), has been implicated in structural plasticity relating to reproductive function. This protein is found in GnRH neurons and hypothalamic astrocytes. The adhesive activity and expression of SynCAM1 in hypothalamic astrocytes is positively regulated by epidermal growth factor B4 (erbB4) receptor signalling (91, 92). Female mice engineered to express a dominant-negative form of SynCAM-1 in astrocytes exhibit delayed vaginal opening and first oestrus, reduced litter size and a disrupted oestrus cycle (91). This suggests astrocytic SynCAM1 mediated structural plasticity is critical for correct reproductive function. Importantly, immunofluorescence and electron microscopy of *post-mortem* human female brain tissue shows that the close anatomical association between GnRH neurons and astrocytes is preserved across species (93).

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In support of a potential role for astrocytes in the regulation of the neuroendocrine control of reproduction, astrocytes are directly responsive to changes in the reproductive cycle. Astrocytes cultured from rat hypothalami express oestrogen receptors and show an increase in intracellular Ca^{2+} and progesterone synthesis in response to oestradiol suggesting they may be involved in integrating hormonal feedback from the gonads (94). Astrocytes in the OVL and POA of young female rats show diurnal changes in morphology on proestrus (the first day of the rodent oestrus cycle); a phenomenon exclusively seen in astrocytes that closely appose GnRH neurons (95). Astrocytes express VAPC2 (Vasoactive intestinal peptide receptor 2), and the diurnal component of these morphological changes is lost when vasoactive intestinal peptide (VIP) is knocked down from the suprachiasmatic nucleus (96). While the function of these changes in astrocyte morphology is not yet clear, it suggests they may be critical in regulating the ability of GnRH neurons to stimulate the proestrus LH surge.

As well as enabling structural reorganisation of neurons, astrocytes can modulate neuronal function by the release of gliotransmitters. One gliotransmitter identified to act on GnRH neurons is prostaglandin E2 (PGE2) (97). PGE2 directly increases the firing rate of GnRH neurons in mice regardless of sex or oestrus stage, an effect blocked by a prostaglandin E2 receptor 2 (EP2R) antagonist. Furthermore, inhibition of astrocytes with the metabolic inhibitor fluorocitrate decreases spontaneous GnRH neuron firing without reducing the response to exogenous PGE2, suggesting astrocytes are the source of PGE2 (98). Neuregulin (NRG) induces PGE2 release via activation of astroglial epidermal growth factor B4 (erbB4) receptors which may promote GnRH secretion. *In vivo* expression of dominant-negative erbB4 receptors in mouse astrocytes (GFAP-DN-erbB4 mice) attenuates the infantile LH surge and delays the onset of puberty in female mice (99). ErbB4-mediated PGE2 release from astrocytes provides excitatory drive to GnRH neurons which may explain the effects of deletion on reproduction (98). Expression of a mutant epidermal growth factor B1 (erbB1) receptor in astrocytes whose intracellular domain is non-functional also causes reproductive dysfunction: irregular oestrus cycles and a reduced proestrus LH surge (100), but it is unclear if this effect is due to decreased PGE2 release. Oxytocin, a hormone critical for the initiation of female puberty, acts at oxytocin receptors on hypothalamic astrocytes to stimulate PGE2

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release and subsequent GnRH secretion (101). A second hormone, namely insulin, also acts directly on astrocytes and deletion of insulin receptors from astrocytes causes reproductive dysfunction in mice. Astrocytes in these animals show lower levels of PGE2 than wild-type controls suggesting insulin acts on hypothalamic astrocytes to stimulate GnRH neurons *via* PGE2 gliotransmission (102). PGE2 also negatively regulates synaptic input to GnRH neurons in an activity dependent manner suggesting astrocytes can decrease GnRH neuronal firing (103). Taken together this work shows that PGE2 release from hypothalamic astrocytes downstream of numerous receptors is important for stimulating GnRH neuron activity and, in turn, normal reproductive function.

In addition to active transmitter release, astrocytes appear to play a critical role mediating peripubertal changes in synaptic glutamate recycling. In the hypothalami of first proestrus rats (compared to juvenile rats), the glutamate synthesis enzyme glutamate dehydrogenase (GDH) is increased while the glutamate metabolic enzyme glutamine synthetase (GS) is decreased (104). This suggests that at first proestrus the hypothalamus is biased towards producing more glutamate which may provide excitatory drive to GnRH neurons for the first LH surge.

In summary, astrocytes in the vicinity of GnRH neurons are indispensable for normal reproductive function since many loss-of-function manipulations result in reproductive deficits. Astrocytes may impact secretion from GnRH neurons by regulating synchronisation via gap junctions, structural remodelling, release of gliotransmitters, and control of glutamate recycling. As such astrocytes can be considered core element of the HPG axis. A schematic summary of the current understanding of how astrocytes may impact the function of the HPG axis can be found in **Figure 4**.

Oxytocin neurons

A second hypothalamic population relevant to neuroendocrine control of reproduction are the magnocellular oxytocin neurons of the supraoptic nucleus (SON). These neurons secrete the hormone oxytocin into the bloodstream to drive lactation and parturition through actions at the breast and uterus respectively. In rats, astrocytes neighbouring oxytocin neurons show a dramatic morphological change, characterised by retraction of processes, during times of high oxytocin secretion such as lactation (105, 106). This increases glutamate availability, presumably because astrocyte processes are not nearby to facilitate reuptake (107). This increase in glutamate availability in the synaptic cleft in turn reduces synaptic input to oxytocin neurons by activating presynaptic metabotropic glutamate receptors (mGluRs), which reduce neurotransmitter release (107). This retraction of astrocyte processes also decreases the availability of D-serine, a glial derived co-agonist of the NMDA receptor, thus impairing glutamatergic plasticity (108). However, the essential nature of astrocytic remodelling in the regulation of lactation is questioned by data indicating that inhibiting this glial remodelling by improving cell-cell adhesion *in vivo* in lactating rats does not produce an abnormal lactation phenotype nor does it disrupt the burst firing of magnocellular neurons characteristic of a lactation signal (109). Thus, astrocytes in apposition to SON oxytocin neurons act to restrict glutamate release and NMDA-receptor activation during lactation, but the functional contribution of these changes remains unclear.

Astrocytes and the neuroendocrine regulation of fluid homeostasis

Arginine Vasopressin (AVP) maintains fluid homeostasis by binding to vasopressin 2 receptors (V2Rs) in the kidney, increasing reabsorption of water back into the blood. AVP is synthesised and secreted from neurons that are found primarily in the paraventricular (PVN), SON and suprachiasmatic (SCN) nuclei of the hypothalamus (110). Magnocellular PVN AVP neurons project to the posterior pituitary gland where AVP is released into the blood or diffusely from dendrites into the hypothalamus through Ca^{2+} dependent exocytosis. Parvocellular PVN neurons, which project to the median eminence, also produce AVP and modulate activity of corticotropes in the anterior pituitary, thus contributing to the

regulation of the hypothalamic-pituitary-adrenal (HPA) axis, which will be discussed in more detail later in this review. AVP is released in response to hyperosmotic stress, with rehydration reducing AVP release. Hypothalamic glutamate concentrations increase during hyperosmotic stress, stimulating SON AVP neurons to fire in a phasic manner leading to AVP release (111-113).

Astrocytes show morphological changes in response to alterations in fluid homeostasis: dehydration reduces GFAP-immunoreactivity in the rodent SON and ventral glial limitans (114-116). Furthermore, *in vitro* AVP increases astrocytic volume (117, 118). In rats, immunoreactivity for c-Fos in the SON, in response to hyperosmotic stress, increases in astrocytes before neurons (119, 120), indicating that astrocytes may be early sensors of dehydration. This is further supported by a study indicating that pharmacological blockade of the gap-junction protein Cx43 prevents osmotic activation of neuronal, but not astrocytic c-Fos, whereas the glial metabolic inhibitor fluorocitrate attenuates c-Fos expression in both neurons and astrocytes (120). Together these data support a critical role for astrocytes in the physiological response to changes in osmolarity.

AVP receptors are expressed on astrocytes (121, 122). Activation of V1bRs and V3Rs by AVP in primary rodent astrocytes *in vitro* results in Ca^{2+} release from intracellular stores, whereas activation of V1aRs leads to increased Ca^{2+} uptake from the extracellular space (123). Both mechanisms result in increased glutamate release from astrocytes, resulting in increased extracellular glutamate concentration (122, 123). Although these studies were conducted in primary rodent astrocytes derived from the cortex and hippocampus, if hypothalamic astrocytes display a similar response, then this increased glutamate availability may be a mechanism by which AVP action on astrocytes modulates neurotransmission.

There is further evidence that astrocytes can impact fluid homeostasis by impacting neuronal firing. *In vitro* AVP increases monocarboxylate transporter (MCT) expression on primary rat astrocytes, increasing lactate release (124), which may increase availability of

lactate as a neuronal fuel source; thus, impacting neuronal firing. In the SON, the amino acid taurine is released during hypo-osmotic cell swelling, reducing firing of AVP neurons via direct binding to glycine receptors (125, 126). This hypo-osmotic regulation of taurine release in the SON is almost completely blocked by glial metabolic inhibitor fluorocitrate, but not by neurotoxic levels of NMDA (126, 127), suggesting that this effect is glial dependent. Within the SON there is also evidence to suggest that astrocytes can modulate GABAergic synaptic inputs via endocannabinoid signalling (128).

Intriguingly, within the PVN the orexigenic hormone ghrelin can directly stimulate firing of AVP neurons, inducing release of AVP which in turn induces ATP release from astrocytes. This subsequently impacts PVN AVP neurons by modulating presynaptic excitatory GABAergic inputs on to these cells (129). These data provide evidence of glial modulation of the activity of PVN AVP neurons and a novel link between the regulation of energy and fluid homeostasis.

In addition to AVP pathway, the renin-angiotensin system is another key player in the neuroendocrine control of fluid homeostasis. Angiotensin II (AngII) is synthesised during dehydration and in response low blood pressure and mediating its physiological effects in this system via type-1 angiotensin II receptors (AT₁-Rs) (130). AngII activates neurons in the subfornical organ (SFO), PVN, and SON, to promote hydration and AVP release (131-133). AT₁-R are expressed on both neurons and astrocytes, with both cell types displaying increases Ca²⁺ signalling in response to AngII treatment (134, 135). In rats, AVP release and sodium intake in response to AngII treatment are blocked by the glial metabolic inhibitor fluorocitrate, indicating that glia may play a key role in AngII/AVP signalling (136). Decreased activity of hypothalamic GLT-1, a glutamate transporter selectively expressed in astrocytes, following AngII treatment has been implicated in increasing activity of PVN neurons and enhancing sympathetic outflow (137). Together these data suggest AngII action via on astrocytes may help to re-establish fluid homeostasis after low blood pressure.

In summary, astrocytes contribute to the regulation of fluid homeostasis via modulation of AVP and renin-angiotensin signalling in the CNS. This appears to be mediated, in part, by direct hormone action on astrocytes. Astrocytes regulate activity of neurons in the PVN and SON by modulating release of gliotransmitters, regulating neurotransmitter availability in the synaptic cleft, and by ensheathment of neurons which is highly plastic dependent on physiological state. A schematic summary of the current understanding of how astrocytes may impact the neuroendocrine regulation of fluid homeostasis can be found in **Figure 5**.

Astrocytes and the neuroendocrine regulation of the stress response: Hypothalamic-pituitary-adrenal axis

The hypothalamic-pituitary-adrenal (HPA) axis is a key neuroendocrine feedback loop regulating the organismal response to stress, encompassing both psychological and physiological stressors. Parvocellular corticotropin-releasing hormone (CRH) and AVP neurons in the hypothalamic PVN project to the median eminence; where CRH and vasopressin are released and transported to the anterior pituitary. There they act synergistically to promote secretion of adrenocorticotropin-releasing hormone (ACTH) from corticotropes. ACTH is then taken up into the blood and in turn acts on the adrenal medulla to promote secretion of cortisol (corticosterone in rodents). Cortisol - acting via the glucocorticoid receptor - provides negative feedback inhibition on the production of ACTH and CRH, by acting on corticotropes and parvocellular PVN neurons respectively. Amplification of HPA-axis activity is provided by adrenaline and noradrenaline release from the adrenal gland in response to activation of the sympathetic nervous system.

Astrocytes, in common with a variety of neural cells, express glucocorticoid and mineralocorticoid receptors (138, 139) and are direct targets of glucocorticoid action. Recent transcriptional analysis indicates that 381 genes are regulated by dexamethasone treatment (a synthetic glucocorticoid) of primary mouse astrocytes (140). Both acute and chronic treatment of mice with corticosterone causes regulation of astrocyte-associated gene expression, reinforcing evidence from *in vitro* studies indicating that astrocytes are directly regulated by glucocorticoids. However, in the case of the *in vivo* studies the impact

is likely a combination of direct (action on astrocytic-glucocorticoid receptors) and indirect effects (action via glucocorticoid receptors on other neural cells, including neurons, which subsequently impact astrocyte activity) (141).

Functional *in vitro* and *in vivo* studies support direct glucocorticoid mediated alterations in astrocyte signalling and morphology. *In vitro*, glucocorticoid treatment induces rapid increases astrocyte Ca^{2+} signalling (142) suggestive of an activation of non-genomic pathways downstream of glucocorticoid receptors. Glucocorticoid treatment of astrocytes in culture also enhances neuropeptide (ANP) release from astrocytes, promotes glutamate recycling, and leads to increases in GFAP-immunoreactivity and rearrangement of F-actin fibres, indicative of cellular stress (143, 144). In contrast, chronic treatment of primary astrocytes in culture with glucocorticoids reduces cellular proliferation and glucocorticoid receptor expression (145, 146). This is recapitulated in an *in vivo* rodent model where astrocyte number is reduced in the frontal cortex by chronic HPA-axis activation through ACTH administration (145) or in response to chronic corticosterone treatment, with or without additional behavioural stress (147, 148). It has been proposed that astrocytes are critical players in the regulation the neurogenesis during stress (149), a phenomenon which may underlie subsequent behavioural adaptations. Importantly, in line with sex differences in the response to stress at the organismal level (150) the response of astrocytes to stress is also sexually dimorphic, which may reflect the intersection of multiple hormonal signalling pathways at the level of the astrocyte (151).

Although not directly studied in this setting, of potential significance for regulation of HPA-axis function, astrocytes are also directly responsive to noradrenaline via receptors expressed on their surface (152). Noradrenaline impacts Ca^{2+} signalling and cellular metabolism in astrocytes (153-155). Neurophysiological studies indicate that within the PVN noradrenaline stimulates ATP release from glia which acts via P2X7 receptor mediated enhancement of cell surface AMPA receptor expression, to enhance glutamatergic tone (156). As such, astrocytes may mediate adrenaline/noradrenaline mediated positive feedback within the HPA-axis. In the context of the regulation of learning and memory, astrocytes have been implicated in mediating some of the effects of the noradrenaline system on this aspect of behaviour (157).

In summary, stress impacts numerous aspects of physiology and behaviour. The potential role of glia in modulating the stress-associated changes in learning and memory have recently been reviewed elsewhere (158). While undoubtedly important players in mediating the response to stress, it remains to be fully clarified whether astrocytes form part of the negative feedback loop that regulates HPA-axis function by directly modulating activity of PVN CRH or AVP neurons. However, given the evidence of astrocytic involvement in regulation of PVN magnocellular AVP neuronal activity in the context of osmotic stress (128, 129) it seems highly likely.

Astrocytes and the regulation of circadian rhythms

To maximise survival most mammals time their peak activity to the presence (diurnal mammals) or absence (nocturnal mammals) of daylight, as appropriate. An internal master clock in the SCN receives information regarding light-dark cycles and uses this to entrain the rest of the body to a roughly 24-hour rhythm (159, 160). The 'hands' of the clock are formed through a molecular transcription-translation negative feedback loop (161). This loop involves many gene and protein interactions; the coordinated activity of CLOCK and brain and muscle aryl hydrocarbon receptor nuclear translocation factor-like protein 1 (BMAL1) to produce PERIOD (PER) and CRYPTOCHROME (CRY) proteins being essential. PER and CRY subsequently inhibit the transcriptional activity of CLOCK/BMAL1, which leads to degradation of PER and CRY, and so restarts the cycle. These clock genes are evolutionarily conserved throughout the animal kingdom, though different homologs exist across species. Found in most mammalian organs, these genes enable cell autonomous rhythmicity that may diverge from that of the SCN (160). Rhythmic expression of clock genes has been identified in astrocytes of the SCN, but how they contribute to circadian regulation is still being fully elucidated.

An abnormal light-dark cycle has a negative impact on health and can cause predisposition to health problems (162). Exposure to constant light or dark alters the expression of GFAP in the SCN, with an increased number of GFAP-immunoreactive cells observed in mice reared in constant light. Conversely, mice reared in constant dark conditions have a reduction in GFAP-immunoreactive cells in the SCN (163). Comparable observations have been made in

rats and hamsters (164-166). This suggests that astrocytes are regulated by changes in the light-dark cycle.

In addition to being responsive to changes in the light-cycle, the activity of SCN neurons can impact astrocytes. Vasoactive intestinal peptide (VIP), one of the key SCN expressed neuropeptides, can entrain the rhythmic expression of *Per2* in cultured astrocytes (167). Furthermore, GFAP-immunoreactive fibre coverage of VIP neurons are light dependent; with increased coverage during the dark phase/circadian night (168). Taken together, these data highlight the complexity of bidirectional control of astrocyte-neuron communication in the SCN.

Astrocytes are emerging as important controllers of the SCN circadian clock. Rhythmic expression of *Bmal1* in astrocytes is important in regulating behaviour and cognition in mice. Inducible-deletion of *Bmal1* from GLAST-positive astrocytes in mice results in altered locomotion, as well as reduced cognitive ability in a novel object recognition task (169). This appears to be mediated in part by alterations in SCN VIP-immunoreactivity and disruption of the rhythmic expression of *Per2* and *Bmal1* in the hippocampus and cortex, demonstrating the importance of astrocytes in regulating circadian rhythms. Of note, in the animals with inducible-deletion of *Bmal1* in GLAST-positive cells, the percentage of non-astrocyte cells in the SCN expressing *Bmal1*-immunoreactivity was also reduced. This suggests that loss of *Bmal1* in GLAST-positive cells impacted cellular expression of this key circadian regulatory gene more widely (169). *In vitro*, knockdown of *Bmal1* mRNA in astrocytes that are co-cultured with neurons is sufficient to impact the rhythmicity of the neuronal expression of clock genes (169). Furthermore, an independent study published contemporaneously demonstrated that knockdown *Bmal1* expression specifically from SCN astrocytes in mice lengthens the circadian period, reflected through altered locomotor activity, and impacts SCN clock gene expression (170). Collectively, these studies suggest that astrocytic expression of *Bmal1* is essential for normal circadian function.

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The molecular mechanisms by which astrocytes interact with neurons to impact circadian rhythms are beginning to be elucidated. Ca^{2+} imaging in cultured SCN slices from the mouse has identified anti-phasic circadian rhythms of Ca^{2+} oscillations between neurons and astrocytes, with astrocytes showing peak activity during the circadian night and neurons the circadian day (171). This is suggestive of a reciprocal activity relationship between the two cell types. Most neurons in the SCN are GABAergic (172), as such GABA as the key neurotransmitter regulating pacemaker activity in the SCN (173). In vitro, pharmacological inhibition of GABA_A receptors in neuron-astrocyte co-cultures is sufficient to prevent astrocyte-induced entrainment of key circadian genes in neurons, including *Per2* (169), suggesting that GABAergic signalling is critical for astrocytic regulation of neuronal circadian function. This *in vitro* evidence is supported by the finding that mice with global inducible knock-out of *Bmal1* in astrocytes display reduced SCN GABA transporter expression and increased cerebrospinal fluid concentrations of GABA, implicating dysregulation of GABA clearance from the extracellular space in the altered circadian phenotype of these animals (169). Indeed, administration of a GABA_A receptor antagonist rescues the dysregulated circadian activity pattern and cognitive deficits in these animals.

Emerging research also supports astrocytic modulation of glutamatergic input as being critical in regulating SCN neurons. Extracellular glutamate levels in the SCN exhibit a circadian pattern, aligned to that of astrocytic Ca^{2+} signalling (171). The activity of glutamine synthetase (GS) is also circadian-dependent (174) and glutamate uptake is considerably hindered in astrocytes cultured from *Clock* and *Per2* deficient mice (175), further supporting a key role for glutamatergic signalling in the regulation of the SCN clock. Brancaccio and colleagues have developed a model for how astrocytic release of glutamate during the circadian night enhances GABAergic tone in SCN neurons; thus, contributing to inhibition of the circuit during that time period and explaining the anti-phasic circadian activity of SCN astrocytes and neurons (171).

In vivo evidence supports a key role for astrocytes in control of SCN periodicity. Floxed CK1 $\epsilon^{\text{tau/tau}}$ mice display a 20h wheel-running activity pattern rhythm in constant darkness. CRE-mediated deletion of CK1 $\epsilon^{\text{tau/tau}}$ specifically from SCN astrocytes is sufficient to restore the behavioural activity in these mice to a 24-hour pattern (171); thus, indicating that astrocytes can impact the intrinsic

activity of the SCN master clock. Indeed, even in the absence of a viable circadian clock (*Cry1/2*-null mice) reintroduction of *Cry1* to SCN GFAP-expressing astrocytes is enough to enable mice to develop an intrinsic rhythmic pattern of behaviour even when maintained in constant darkness (176). This is mediated, at least in part, via astrocyte-driven glutamatergic regulation of SCN neuronal activity (176). These seminal studies indicate that SCN astrocyte-communication plays a prominent role in maintaining circadian function. A schematic summary of the current understanding of how astrocytes may impact the control of circadian rhythms by the SCN can be found in **Figure 6**.

Summary and conclusions

Astrocytes play a fundamental role in monitoring and responding to changes in hormones and nutrients as they penetrate the brain or are released locally. The structural apposition of astrocytes to both cerebral blood vessels (together with pericytes) and neurons allows coupling of these cues to changes in the activity of astrocytes and subsequently neurons. Over the past decade or so, the generation of astrocyte-specific genetic manipulations in mice has permitted the assessment of glial roles in regulating whole body physiology, including (but not limited to) regulation of food intake, glucose homeostasis, reproduction, fluid balance, breathing and blood pressure regulation. Emerging technological advancements permitting more spatially and temporally-targeted manipulation of astrocytes will enable previously unknown astrocyte functions to be described. Going forward, better classification of astrocytes into specific subtypes, akin to those of neurons (i.e. glutamatergic and GABAergic neurons) is fundamentally required and will allow yet more detailed examination of the astrocyte contribution to neuroendocrine function. This will clearly be challenging given that astrocytes are highly plastic cells, reacting and adapting to new physiological and pathophysiological environments. Importantly, assessment of astrocyte function and the gliotransmitters they release (and their receptors) may expose potential therapeutic targets for treatment of significant public health challenges including obesity, diabetes and endocrine disorders such as hypothyroidism and hypogonadism.

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Figure Legends

Figure 1: Schematic overview of the proposed mechanisms by which astrocytes can impact neuroendocrine systems. (1) As a key component of the blood-brain barrier (BBB), astrocytes may impact entry of circulating factors, including hormones and nutrients in to the CNS. (2) Like neurons, astrocytes are direct targets of hormone action in the CNS via receptors expressed on their surface. (3) Astrocytes are a source of bioactive signalling molecules including gliotransmitters, and neurosteroids. Astrocytes also regulate neurotransmitter availability in the synaptic cleft through reuptake and recycling of neurotransmitters. (4) Morphological changes in astrocytes results in ensheathment of synapses which impacts synapse stability and extra-synaptic communication.

Figure 2: Simplified schematic summary of mechanisms by which hypothalamic astrocytes regulate neuroendocrine control of feeding. (1) Astrocytes of the hypothalamus express receptors for key homeostatic hormones, including leptin and ghrelin; (2) Deletion of leptin receptors and changes in diet alter the synaptic ensheathment of neighbouring neurons which control feeding; (3) Chronic leptin or ghrelin administration, and changes in food intake, change the expression of glial glutamate and glucose transporters in the medial basal hypothalamus; (4) Hypothalamic astrocytes show inflammatory responses and morphological plasticity in response to intake of a high-fat diet and manipulation of these inflammatory pathways (namely the NF- κ B pathway) in astrocytes modulates feeding and can be protective against diet-induced obesity.

Figure 3: Simplified schematic summary of mechanisms by which astrocytes regulate glucose homeostasis. (1) Astrocytes express glucose transporter (GLUT) 1 and 2 and their expression is involved in stimulating the counterregulatory response (CRR) to hypoglycaemia; (2) Reduced expression of glial glutamate transporters after recurrent hypoglycaemia disrupts glutamatergic neurotransmission necessary for the appropriate CRR; (3) Astrocytes store glycogen and use this to provide fuel to neurons during glucose deprivation, however glycogen supercompensation following recurrent hypoglycaemia may blunt the CRR; (4) Inflammation and astrogliosis are observed in mouse models of diabetes and modulation of astrocyte NF- κ B signalling is protective against diet-induced glucose intolerance; (5) By releasing gliotransmitters, altering physical contact, and reuptake of neurotransmitters, astrocytes modulate neurons in the brainstem and hypothalamus to control neuronal responses to changes in glucose availability.

Figure 4: Simplified schematic summary of mechanisms by which hypothalamic astrocytes regulate neuroendocrine control of reproduction. (1) Astrocytes of the hypothalamus express receptors for numerous neuropeptides and hormones including oestradiol, vasoactive intestinal polypeptide (VIP), neuregulin, and oxytocin; (2) Stimulation of certain receptors on astrocytes (namely epidermal growth factor B4 [erbB4], oxytocin, and insulin receptors) leads to release of prostaglandin E2 (PGE2) which acts on prostaglandin E2 receptor 2 (EP2R) on GnRH neurons to increase firing and GnRH secretion; (3) Astrocytes are responsible for the reuptake and synthesis of glutamate and the levels of proteins required for this process vary throughout reproductive maturation; (4) Gap junction connections between astrocytes and GnRH neurons may allow for synchronous firing and hormone release between spatially separated neurons. The net effect of these mechanisms is to modulate GnRH secretion, a master regulator of reproductive function.

Figure 5: Simplified schematic summary of mechanisms by which astrocytes modulate the neuroendocrine regulation of fluid homeostasis. (1) Astrocytes in the hypothalamus express receptors for arginine vasopressin (AVP; V3R, V1aR, and V1bR) and angiotensin II (AngII; AT₁R). During hyperosmotic events, AVP and AngII activate intracellular signalling pathways in astrocytes which increase intracellular Ca²⁺; (2) Increases in intracellular Ca²⁺ lead to increased glutamate release from astrocyte which may act on neurons to drive phasic firing and AVP release; (3) Taurine is released from astrocytes under hypo-osmotic

stress which can inhibit AVP neurons by activating neuronal glycine receptors; (4) Astrocytes of the supraoptic nucleus show morphological changes and c-FOS expression in response to dehydration and *in vitro* astrocytes swell in response to hypo-osmotic stress. Thus, astrocytes can sense and respond to changes in fluid homeostasis.

Figure 6: Simplified schematic summary of mechanisms by which hypothalamic astrocytes regulate the circadian ‘master’ clock of the suprachiasmatic nucleus. The suprachiasmatic nucleus (SCN) within the hypothalamus is responsible for entraining daily rhythms to the light-dark cycle. (A) Within the SCN, astrocytes have rhythmic oscillations in Ca^{2+} anti-phasic with those seen in neurons: astrocytes showing peak activity during the circadian night and neurons the circadian day. (B) Within SCN the bi-directional communication between neurons and astrocytes is important for maintaining integrity of the master circadian clock: (1) Astrocytic recycling of glutamate and GABA contributes to regulation of SCN neuron activity; (2) Vasoactive intestinal peptide (VIP), a key neuropeptide transmitter in the SCN, can entrain rhythmic expression of ‘circadian clock genes’ in astrocytes; (3) Astrocyte ensheathment of SCN VIP neurons is light dependent, with increased coverage during the circadian night.

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